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PENSOFT

First record of *Leiostracus demerarensis* (L. Pfeiffer, 1861) from Brazil (Gastropoda, Orthalicoidea), with a taxonomic reassessment

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Abstract

We report the first Brazilian record of *Leiostracus demerarensis* (L. Pfeiffer, 1861) from Pará and Maranhão states. The distribution of this species now comprises Guyana, Suriname, French Guiana, and northern Brazil. Furthermore, given the uncertainty in generic and familial allocation of this species (either *Bostryx* Troschel, 1847, Bulimulidae, or *Leiostracus* Albers, 1850, Simpulopsidae), we used the barcoding segment of the COI gene to ascertain its classification in Simpulopsidae, retaining it as *Leiostracus demerarensis*. Moreover, *Simpulopsis luteolus* (Ancey, 1901) is also reported for the first time from Pará state.

Keywords

Bostryx, Bulimulidae, Maranhão, Pará, Simpulopsidae, Stylommatophora.

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Introduction

Mollusks, especially freshwater and terrestrial, have been widely recognized as one of the most vulnerable animal groups on the planet. These animals are under constant pressure as their environments are increasingly altered and exploited by humans, resulting in very high extinction rates. In this gloomy scenario, many species are vanishing before they had the chance to be studied (Lydeard et al. 2004; Régnier et al. 2009). The Brazilian land snails are not an exception to this rule, especially in face of recent political changes and the consequent increasing rates of deforestation (Nature Editorials 2018), which makes studying these animals an urgent task (Salvador and Simone 2015; Salvador 2019).

Recent ornithological expeditions to Pará state, northern Brazil, also acquired several land snail specimens. Among these, we identified representatives of *Leiostracus demerarensis* (L. Pfeiffer, 1861), a species previously unrecorded from Brazil. Herein, we report these new records and use the barcoding segment of the COI gene to ascertain the classification of this species, which is controversial in the literature.

Methods

The specimens studied herein were collected by ACDL in two localities in Pará state, Brazil (Fig. 1): (1) Jacareacanga municipality, Thaimaçu Lodge property, where live specimens were found over dead leaves on

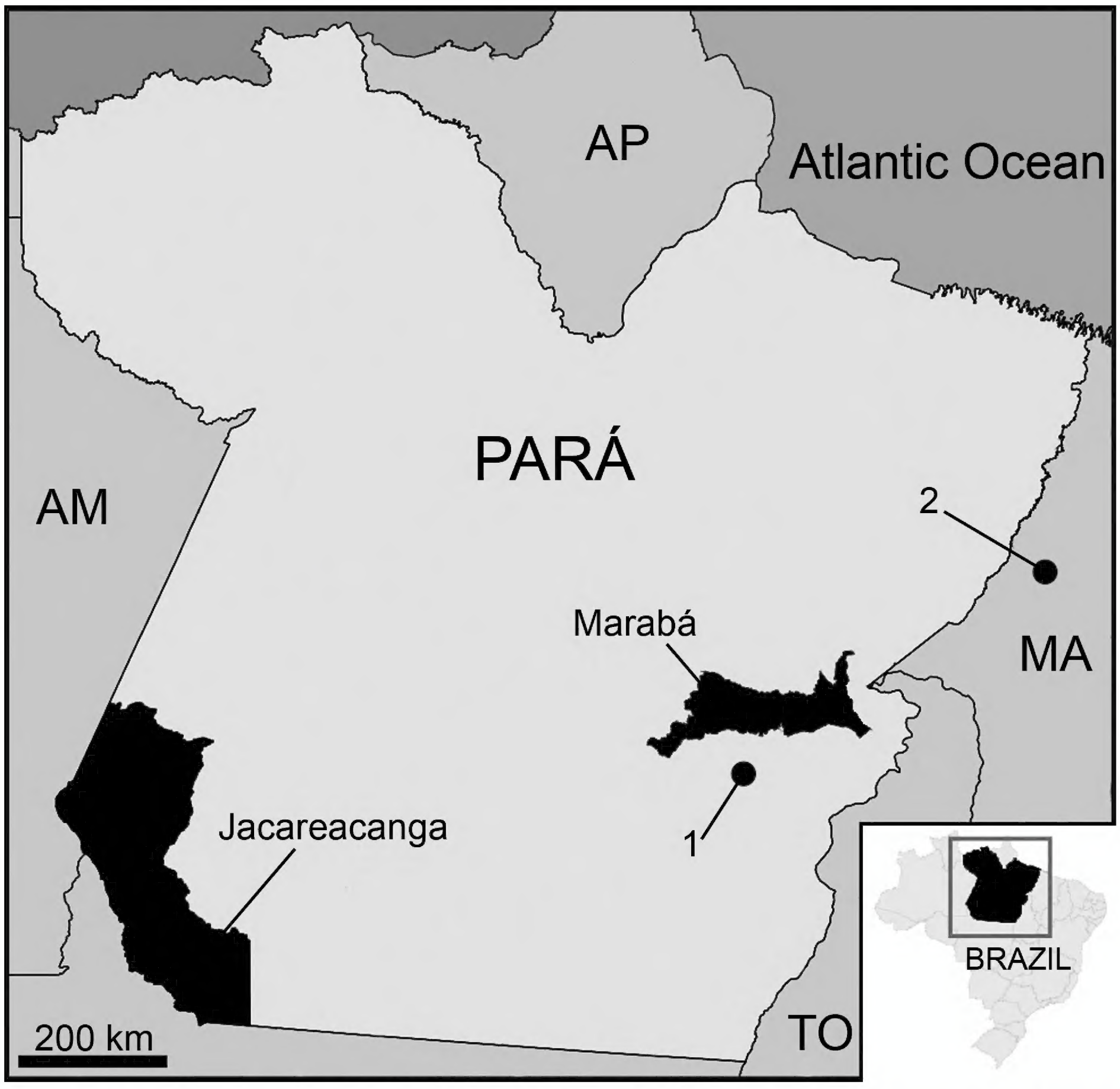

Figure 1. Map of Pará state showing the municipalities where *Leiostracus demerarensis* was collected. The numbers indicate localities where the species was observed, but not collected: 1, Canaã dos Carajás; 2, Bom Jardim (Maranhão state).

the leaf litter and on narrow branches of small bushes, at a maximum height of 1.5 m. The locality was a second-growth forest with the understory dominated by a dense bamboo thicket (*Guadua* sp.). (2) Marabá municipality, Vila Itainópolis, where live specimens were found on the trunks of trees and bushes, from 1 to 1.5 m high, on a dense and humid secondary "terra firme" forest fragment. Specimens were fixed and preserved in ethanol 98%, and deposited in the following malacological collections: CMRP, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (Ribeirão Preto, Brazil); MZSP, Museu de Zoologia da Universidade de São Paulo (São Paulo, Brazil); NMNZ, Museum of New Zealand Te Papa Tongarewa (Wellington, New Zealand). Additional specimens were observed alive by ACDL, but not collected, in Canaã dos Carajás municipality (Pará state) and Bom Jardim municipality (Maranhão state).

One randomly selected adult specimen from each locality had a small section of the foot clipped for molecular study. DNA extraction was conducted with QIAGEN DNeasy® Blood & Tissue Kit, following standard protocol. The marker targeted was the barcoding fragment of the mitochondrial COI gene (primers LCO and HCO described by Folmer et al., 1994), with circa 650 bp. PCR protocol as follows: (1) initial denaturation at 96 °C (2 minutes); (2) denaturation at 94 °C (30 seconds); (3) annealing at 48 °C (1 minute); (4) extension at 72 °C (2 minutes); (5) repeat steps (2) to (4) 34 times, for a total of 35 cycles; (6) final extension at 72 °C (5 minutes). The PCR products were quantified via agarose gel electrophoresis, cleaned following standard ExoSAP-IT™ protocol (Affymetrix Inc.), and Sanger sequenced. Resulting sequences were assembled and quality-checked in Geneious Prime (version 2019.0.3,

Biomatters Ltd), and uploaded to NCBI GenBank under the accession numbers MN175957 (NMNZ M.328328) and MN175958 (NMNZ M.328329).

Additional COI sequences from 31 related orthalicoid species were obtained from the work of Breure and Romero (2012), to which we included two species of Hygrophila as outgroup (see Appendix for full list and GenBank accession numbers). The sequences were 654 bp long, with the exception of one Hygrophila that presented an indel, totaling 669 bp. Sequences were aligned in Geneious Prime using the MUSCLE plugin (Edgar, 2004) with default settings (i.e., optimized for accuracy). The resulting alignment was manually proofed for errors. A tree was built in Geneious by Bayesian Inference using the MrBayes plugin (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) with the following settings: HKY85 substitution model, 200,000 burn-in length, and 1,100,000 iterations.

Results

New records. BRAZIL • 18 specimens; Pará state, Jacareacanga municipality, Thaimaçu Lodge property; 09°05′45″S, 056°37′12″W; Oct. 2017; A.C. De Luca leg.; over dead leaves on leaf litter; GenBank: MN175957; CMRP 861 (12 specimens), MZSP 152044 (1), MZSP 152045 (1), NMNZ M.328328 (4). BRAZIL • 4 specimens; Pará state, Marabá municipality, Vila Itainópolis; 05°39′30″S, 049°28′54″W; Nov. 2016; A.C. De Luca leg.; on the trunks of trees and bushes; GenBank: MN175958; CMRP 867 (2 specimens), NMNZ M.328329 (2).

Additional specimens were observed alive (Fig. 1) in: (1) Canaã dos Carajás municipality (Pará state), on the leaves of bushes, around 1.5 m high, on a transitory forest between the "terra firme" and "canga" types of forest. The latter type is characterized by open grassy vegetation growing on rocky iron-rich soils (Mota et al. 2018). (2) Bom Jardim municipality (Maranhão state), specimens were only found as empty shells on the ground in "terra firme" forest, characterized by sandy soil and an abundance of Babaçu palm trees (*Attalea* sp.).

Other specimens collected in Jacareacanga include representatives of *Aperostoma fultoni* Bartsch & Morrison, 1942 (vouchers CMRP 862, CMRP 864), *Drymaeus ribeiroi* Ihering, 1915 (CMRP 860, CMRP 863), *Drymaeus* sp. (not collected), *Psadara rugifera* (Dohrn, 1882) (CMRP 859), and *Streptartemon extraneus* Haas, 1955 (CMRP 865). Other specimens collected in Marabá include representatives of *Aperostoma blanchetiana* (Moricand, 1826), *Corona duckei* Ihering, 1915, *Euglandina irakita* Jardim, Abbate & Simone, 2013, *Helicina laterculus* F.C. Baker, 1914, *Simpulopsis luteolus* (Ancey, 1901) (CMRP 884), and *Ringicella ringens* (Linnaeus, 1758). All these species are already known from Pará state (Simone 2006), with the exception of *Simpulopsis luteolus* (Fig. 2), which represents the first report of the species for the state. Otherwise, this species was only known from Goiás state to the south (Simone 2006).

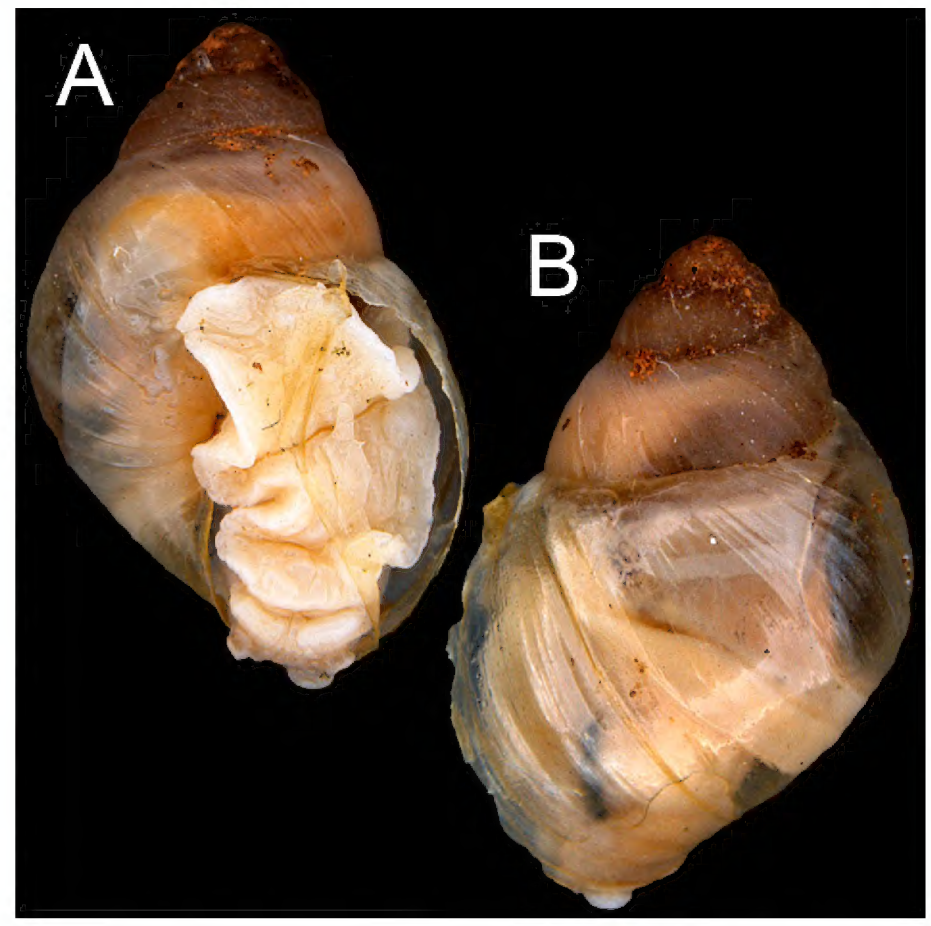

Figure 2. *Simpulopsis luteolus*, CMRP 884 (shell height = 9.6 mm, width = 6.0 mm), from Marabá. **A.** Apertural view. **B.** Dorsal view.

Identification. The shells of the present specimens of *Leiostracus demerarensis* (Fig. 3) are indistinguishable from the lectotype of the species (Natural History Museum, London, UK, NHMUK 1975501, designated by Breure 1978; see also Breure and Ablett 2015: figs 21vi–viii), as well as consistent with other published images of the species (Massemin et al. 2009; Muratov and Gargominy 2011). The most important features for identification are the protoconch sculpture (numerous delicate spiral striae) and whorl count (ca 1½ whorls), the relatively small shell size for the genus, the peripheral angulation on the median portion of the body whorl, and the color pattern (Pilsbry 1897–1898; Muratov and Gargominy 2011). For a description of the species anatomy, see Muratov and Gargominy (2011).

Discussion

The present species was originally described as *Bulimus demerarensis*, with the type locality at Demerara, Guyana (Pfeiffer 1861). A second species, *Drymaeus* (*Leiostracus*) *ruthveni* H.B. Baker, 1926, described from Dunoon, Guyana (Baker 1926), was later brought into its synonymy (Breure 1978; Muratov and Gargominy 2011).

Bulimus demerarensis has been historically classified in *Leiostracus* Albers, 1850, especially due to its small size and the peripheral angulation on the body whorl of the shell, typical of the genus (Zilch 1960). However, its protoconch sculpture (numerous delicate spiral striae) is consistent with both *Leiostracus* and some species presently assigned to *Bostryx* Troschel, 1847 (Muratov and Gargominy 2011).

In a recent revision, however, Muratov and Gargominy (2011) analyzed the anatomy of a specimen from French Guiana and transferred the species to *Bostryx* due to the lack of one diagnostic feature of *Leiostracus*:

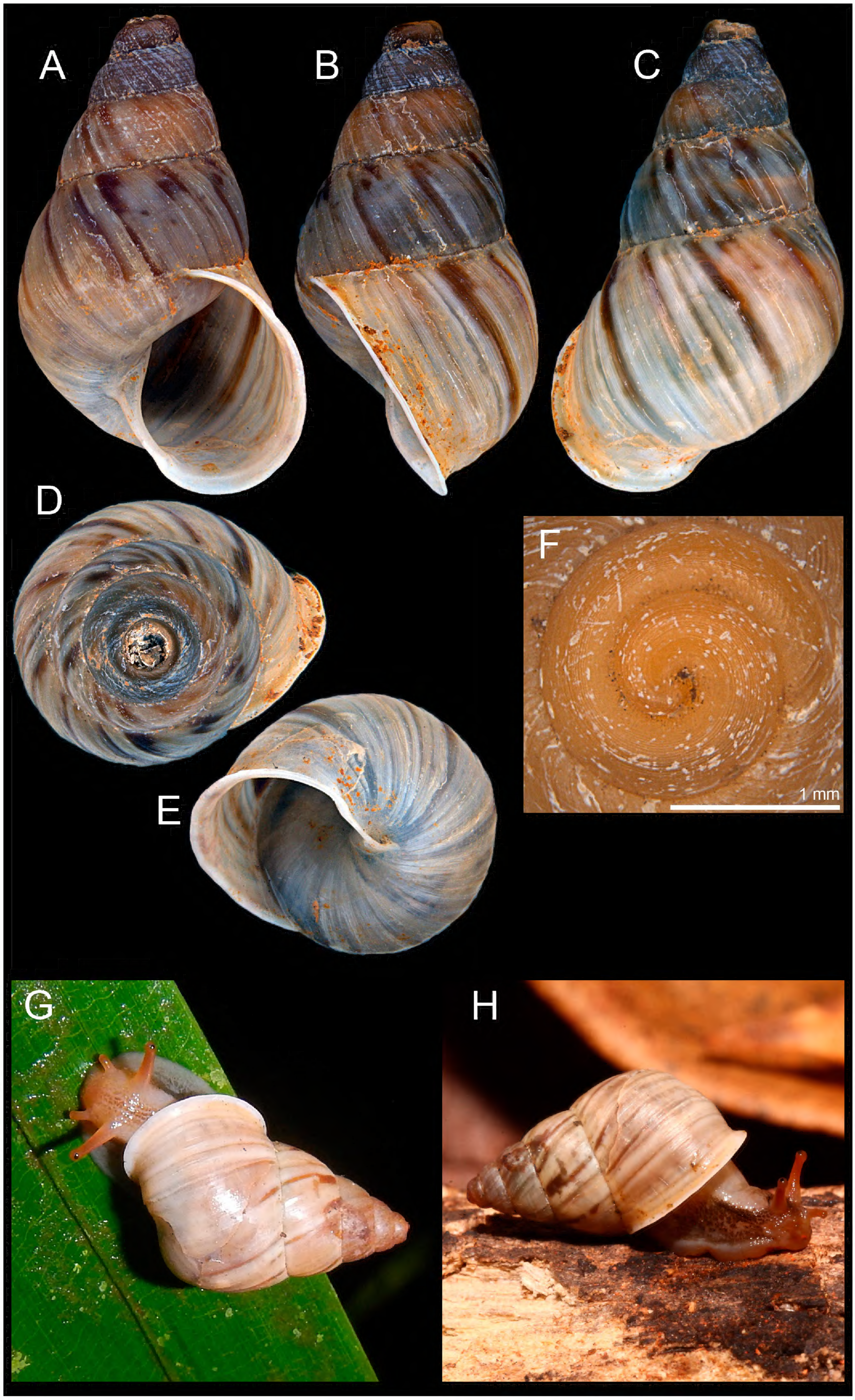

Figure 3. *Leiostracus demerarensis*. **A–E.** CMRP 861, #1 (shell height = 14.0 mm, width = 9.3 mm) from Jacareacanga. **A.** Apertural view **B.** Lateral view. **C.** Dorsal view. **D.** Apical view. **E.** Umbilical view. **F.** CMRP 861, #2, protoconch detail. **G, H.** Live individual *in situ* (Marabá), not collected.

the "division of the spermathecal duct into an enlarged distal part and a slender proximal part" (Muratov and Gargominy 2011: 614; see also Breure 1978, 1979). Nevertheless, Breure and Ablett (2015) considered this evidence insufficient for the reclassification and maintained the classification in *Leiostracus*. These authors argued that *Bostryx* and *Leiostracus* belong to two distinct orthalicoid families (Bulimulidae and Simpulopsidae, respectively) according to the most recent molecular studies (Breure and Romero 2012) and concluded that further evidence was needed to support the new classification of *B. demerarensis*. Furthermore, *Bostryx* has an Andean distribution, with a single species reported from Venezuela (Breure 1979; Muratov and Gargominy 2011), which is geographically removed from the distribution of *Leiostracus demerarensis*.

We addressed this matter by sequencing the barcoding segment of the COI gene and including the present specimens of *L. demerarensis* in the dataset of the Orthalicoidea phylogeny published by Breure and Romero (2012). The results of our Bayesian analysis showed that the species is nested in Simpulopsidae, forming a clade with the representative of this family (genus *Simpulopsis* Beck, 1837) with a posterior probability of 0.97 (Supplementary Material; the two sequenced *L. demerarensis* specimens clustered together with a posterior probability of 1.0). Given this new insight and the species morphological features (small size, peripheral angulation on the body whorl, and protoconch sculpture), our work supports the allocation in Simpulopsidae. As such, we retain the classification as *Leiostracus demerarensis*.

The previously known distribution of the species comprises Guyana, Suriname, and French Guiana (Massemin et al. 2009; Muratov and Gargominy 2011). The new records reported herein extend this species' distribution to Brazil, with four distinct localities in two neighboring states (Fig. 1): Jacareacanga, Canaã dos Carajás and Marabá (Pará state); Bom Jardim (Maranhão state). These localities, while still belonging to the vast Amazon biome, are outside the Guyana Shield and south of the Amazon River, indicating that the species is widespread over a potential geographical barrier. The two Brazilian specimens sequenced show some genetic divergence from one another (Supplementary Material), but it is presently not possible to know the divergence, if any, from the northern Guyanese population.

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Authors' Contributions

ACDL collected the specimens. DCC prepared the figures. RBS conducted genetic analysis. All authors contributed to species identification and writing of the manuscript.

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Supplementary Material

Figure S1. Bayesian inference tree of COI marker.

Appendix

Below are listed the COI sequences of other Orthalicoidea species, obtained from the work of Breure and Romero (2012), used to ascertain the classification of the species studied herein. The list is organized by family and species (in alphabetical order), followed by the respective GenBank accession number. **Bulimulidae:** *Bostryx agueroi* Weyrauch, 1960 JF514623; *Bostryx bilineatus* (Sowerby I, 1833) JF514637; *Bostryx edmundi* Breure and Neubert, 2008 JF514622; *Bostryx longispira* Weyrauch, 1960 JF514624; *Bostryx strobeli* Parodiz, 1956 JF514636; *Bostryx superbus* Weyrauch, 1967 JF514621; *Bulimulus diaphanus* (L. Pfeiffer, 1854) JF514633; *Bulimulus guadalupensis* (Bruguière, 1789) JF514630; *Bulimulus hummelincki* (Breure, 1974) JF514629; *Bulimulus sporadicus* (d'Orbigny, 1835) JF514632; *Bulimulus tenuissimus* (d'Orbigny, 1835) JF514631; *Drymaeus inusitatus* (Fulton, 1900) JF514648; *Drymaeus laticinctus* (Guppy, 1868) JF514646; *Drymaeus multifasciatus* (Férussac, 1821) JF514647; *Drymaeus vexillum* (Broderip, 1832) JF514625; *Naesiotus quitensis* (L. Pfeiffer, 1848) JF514635; *Naesiotus stenogyroides* (Guppy, 1868) JF514650; *Neopetraeus tessellatus* (Shuttleworth, 1852) JF514627; *Rabdotus alternatus* (Say, 1830) JF514638; *Scutalus chiletensis* Weyrauch, 1967 JF514628. **Odontostomidae:** *Clessinia cordovana cordovana* (L. Pfeiffer, 1856) JF514618; *Clessinia cordovana stelzneri* (Doering, 1875) JF514617; *Clessinia pagoda* Hylton Scott, 1967 JF514613; *Cyclodontina guarani* (d'Orbigny, 1835) JF514619; *Plagiodontes multiplicatus* Doering, 1874 JF514620; *Spixia pervarians* (Haas, 1936) JF514614; *Spixia philippii* (Doering, 1875) JF514612; *Spixia popana* (Doering, 1877) JF514616; *Spixia tucumanensis* (Parodiz, 1941) JF514615. **Simpulopsidae:** *Leiostracus perlucidus* (Spix, 1827) JF514640; *Simpulopsis decussata* L. Pfeiffer, 1856 JF514639. The sequences of the two outgroup taxa were obtained from GenBank: *Acroloxus lacustris* (Linnaeus, 1758) AY282581 (Acroloxidae); *Planorbis planorbis* (Linnaeus, 1758) EF012175 (Planorbidae).